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Chapter 5

Interaction Between Factor VIII and LDL Receptor-related Protein: Modulation of Coagulation?

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Chapter 1

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Summary

Recent reports suggest that the multifunctional receptor Low density lipoprotein receptor-Related Protein (LRP) may contribute to the regulation of blood coagulation by mechanisms that differ from the simple removal of protease/inhibitor complexes from the circulation. This possibility became apparent from the observation that LRP is involved in down-regulation of Tissue Factor expression at the surface of monocytes and fibroblasts. Furthermore, coagulation Factor VIII and activated Factor IX (Factor IXa) have been identified as proteins that are able to bind to LRP. In the present review, the potential contribution of LRP to the regulation of the coagulation cascade through these novel pathways will be discussed, with particular reference to the interaction between LRP and coagulation Factor VIII.

Blood Coagulation Factor VIII

Bleeding tendencies associated with the functional absence of coagulation Factor VIII are known as hemophilia A (Sadler and Davie 1987). This disease, which has been recognized for several centuries, is an X-linked disorder affecting 1-2 in 10,000 males. The activated derivative of Factor VIII serves as a cofactor for the serine protease Factor IXa in the Factor X-activating complex of the intrinsic coagulation pathway (for recent reviews on Factor VIII see Kaufman et al. 1997; Lenting et al. 1998).

The gene of Factor VIII is located at the tip of the long arm of the X chromosome and spans over 180 kb, comprising 26 exons that encode a polypeptide chain of 2351 amino acids. The polypeptide includes a signal peptide of 19 and a mature protein of 2332 amino acids. Analysis of the amino acid sequence revealed the presence of a discrete domain structure within Factor VIII. Based on internal homology, these domains are arranged in the sequence A1-a1-A2-a2-B-a3-A3-C1-C2 (Figure 1). The A domains display approximately 30 % homology to each other, and to homologous A domains found in the copper-binding protein ceruloplasmin and coagulation Factor V. The A domains are bordered by short segments (a1, a2 and a3) that are enriched in glutamate and aspartate residues, the so-called acidic regions. Factor VIII C domains are structurally related to those present in Factor V, and various other proteins like the lipid-binding lectin discoidin I. The B domain is unique in that it exhibits no significant homology with any other known protein.

Due to endoproteolytic processing, Factor VIII circulates in plasma predominantly as a heterodimeric protein consisting of a metal ion-linked heavy and light chain (Figure 1). The heavy chain (90-220 kDa) contains the A1-a1-A2-a2-B domains and is heterogeneous as a result of limited proteolysis within the B domain. The light chain (80 kDa) comprises the domains a3-A3-C1-C2. In plasma, Factor VIII is present as an inactive precursor that is tightly associated with its carrier protein von Willebrand Factor (vWF) through the amino- and carboxyterminal ends of Factor VIII light chain. Factor VIII is converted into its active form by thrombin-mediated proteolysis in both Factor VIII heavy chain and light chain. The light chain is cleaved between a3 and A3 domain, while the heavy chain is cleaved between a1 and A2 domain and between a2 and B domain. The final activated product, Factor VIIIa, thus consists of the metal ion-linked heterodimer A1-a1/A3-C1-C2 that is associated with the heavy chain derived
A2-a2 portion, whereas the B domain and the a3 segment have been removed (Figure 1). Release of this a3 segment is associated with the loss of high affinity binding to vWF. Binding of Factor VIII to vWF serves an important role in Factor VIII physiology. Not only is the Factor VIII heterodimeric structure stabilized upon association, but vWF also prevents Factor VIII from binding to other proteins. It has previously been established that Factor VIII may interact with a variety of proteins, including activated protein C, Factor Xa and Factor IXa. Recently, Factor VIII was shown to interact with the multifunctional receptor LRP as well (Lenting et al. 1999; Saenko et al. 1999).

![Diagram of Factor VIII](image)

**Fig. 1: Domain structure of Factor VIII.** Factor VIII circulates in plasma predominantly as a heterodimeric protein consisting of a heavy and light chain that are linked in a divalent metal ion-dependent interaction between A1 and A3 domain. The heavy chain contains the A1-a1-A2-a2-B domains and the light chain comprises the domains a3-A3-C1-C2. Factor VIII is tightly associated with vWF through the amino- and carboxyl-terminal ends of Factor VIII light chain. Upon thrombin activation the Factor VIII heterodimer is cleaved in both heavy and light chain (positions indicated by arrows) resulting in releases of the B domain and the a3 segment. Removal of this a3 segment is associated with the loss of high affinity binding to vWF. Within Factor VIIIa trimer binding sites for Factor IXa, activated Protein C (aPC), phospholipids, and LRP are indicated.

**Low Density Lipoprotein Receptor-Related Protein**

LRP, also known as the α2-macroglobulin receptor, is a 600-kDa membrane glycoprotein that is a member of the low density lipoprotein (LDL) receptor family of endocytic receptors (for recent reviews on LRP see Gliemann 1998; Neels et al. 1998). This growing receptor family includes the low density lipoprotein receptor, LRP2 (also
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known as gp330 or megalin), very low density lipoprotein receptor, and apolipoprotein E receptor 2. Common structural features of these receptors include: 1) clusters of class A cysteine-rich ligand binding repeats called LDL receptor class A (LDLRA) domains, 2) epidermal growth factor-like domains, 3) spacer regions that include Tyr-Trp-Thr-Asp repeats, 4) a single transmembrane domain, and 5) a carboxyl-terminal cytoplasmic tail with 1-3 copies of the Asn-Pro-Xxx-Tyr motif, which serve as potential endocytosis signals. The gene coding for LRP covers about 92 kb and includes 89 exons. The 15-kb LRP cDNA encodes a protein that consists of 4525 amino acids. LRP is characterized by the presence of 31 LDLRA repeats, which are arranged in four distinct clusters (denoted clusters I, II, III, and IV) with 2, 8, 10, and 11 LDLRA domains, respectively. Clusters II and IV have been shown to constitute the ligand binding domains (Willnow et al. 1994; Neels et al. 1999). LRP is synthesized as a single polypeptide chain and is cleaved in the trans-Golgi network by the endopeptidase furin. Furin-mediated proteolysis results in a 515-kDa fragment that contains the ligand binding domains and an 85-kDa fragment comprising the transmembrane and cytoplasmic domains. The subunits remain associated in a noncovalent fashion as they are routed to the cell surface. Routing of LRP to the cellular surface is mediated by its chaperone protein, the 39 kDa Receptor-Associated Protein (RAP). RAP is essential for optimal folding of LRP, and blocks premature, intracellular binding of LRP to its ligands (Bu and Schwartz 1998). RAP has proved to serve as a valuable tool in studies directed to interactions between LRP and its ligands.

LRP is abundantly present in various tissues like liver, placenta, lung, and brain and is expressed in an array of cell types, including parenchymal cells, Kupffer cells, neurons, astrocytes, smooth muscle cells, monocytes, adipocytes, and fibroblasts. Also commonly used cell lines like Chinese hamster ovary (CHO) cells express LRP (FitzGerald et al. 1995). LRP is involved in the transport of ligands from the cell-surface to the endosomal degradation pathway, a process which requires the presence of calcium ions (Moestrup et al. 1990). Furthermore, LRP has recently been proposed to contribute to the transcytosis of lactoferrin through the blood-brain barrier (Fillebeen et al. 1999), and appears to be important in various cellular signaling processes (Willnow et al. 1999). The multifunctional nature of LRP is underscored by the observation that targeted disruption of the gene coding for LRP in the mouse arrested the development of LRP−/− embryos around the implantation stage (day 13 of development) (Herz et al. 1992). At present, a remarkable spectrum of structurally-unrelated ligands has been identified. These include apolipoproteins, lipases, proteases, protease/inhibitor complexes, Kunitz-type inhibitors, matrix proteins, and several others (Gliemann 1998; Neels et al. 1998; Willnow et al. 1999). This broad range of ligands and properties suggests a role for the receptor in diverse physiological and patho-physiological processes ranging from lipoprotein metabolism, fibrinolysis, cell growth and migration to atherosclerosis, tumor metastasis, and Alzheimer’s disease. In addition, LRP might play a role in hemostasis as will be discussed below.

Interaction Between Factor VIII and LRP

The mechanism by which Factor VIII is removed from the circulation is poorly understood. The possibility that LRP may serve as a clearance receptor for Factor VIII
is recently reported by two different research groups (Lenting et al. 1999; Saenko et al. 1999). By using purified proteins, we found that the interaction between Factor VIII and LRP is reversible and both dose- and calcium-dependent. In addition, the interaction is efficiently inhibited in the presence of the LRP-antagonist RAP. The kinetic parameters, describing complex assembly between Factor VIII and LRP, indicate that Factor VIII binds to LRP with moderate affinity (affinity constant ≈ 60 nM). This value is close to that reported by Saenko and coworkers (affinity constant = 116 nM), and in the same range as described for most of the other ligands for LRP, like hepatic lipase (52 nM) (Kounnas et al. 1995a), β-amyloid precursor protein (80 nM) (Kounnas et al. 1995b), two-chain urokinase (60 nM) (Kounnas et al. 1993) and plasminogen activator inhibitor-1 (35 nM) (Horn et al. 1995). It is important to note, however, that the physiological concentration of Factor VIII (approximately 0.5 nM) is 100-fold below the affinity constant of the interaction between Factor VIII and LRP. It seems conceivable therefore that in vivo complex formation between Factor VIII and LRP is fully driven by local concentrations of the receptor. Alternatively, a mechanism may exist that concentrates Factor VIII at cellular surfaces enriched in LRP.

The interaction between LRP and Factor VIII was also tested using CHO cells, which constitutively express LRP. CHO-cells efficiently degrade 125I-labeled Factor VIII, a process which is reduced approximately 70 % in the presence of RAP. A similar inhibition was found using an anti-LRP polyclonal antibody preparation (Lenting et al. 1999a). These findings indicate that LRP contributes to the cellular uptake of Factor VIII. This is further supported by experiments using cell lines which lack LRP. These LRP-deficient cell lines, both mouse embryonic fibroblasts and CHO cells, degrade Factor VIII 2-3 fold less efficiently as compared to their LRP-expressing counterparts (Figure 2) (Saenko et al. 1999). In summary, these data are compatible with a mechanism in which LRP contributes to the binding and transport of Factor VIII to the intracellular degradation pathway.

**Fig. 2: Cellular degradation of Factor VIII and its light chain in the presence and absence of LRP.** Degradation of either 125I-Factor VIII or 125I-Factor VIII light chain was measured in time on both LRP expressing and LRP deficient CHO cells. Wells containing 2 x 10⁵ LRP expressing CHO cells (●) or LRP deficient CHO cells (○) were incubated with either 20 nM 125I-Factor VIII (left panel) or 20 nM 125I-Factor VIII light chain (right panel) for selected time intervals at 37 °C, and the amount of degraded material was determined. Degraded material is defined as radioactivity present in the overlaying medium that is soluble in 10 % trichloroacetic acid.
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Regions Involved in LRP/Factor VIII Complex Assembly: Factor VIII Light Chain

Following the identification of Factor VIII as an LRP ligand, research was directed to the identification of those regions which contribute to assembly of the LRP/Factor VIII complex. By using ligand blotting, we found that the isolated light chain of Factor VIII is able to bind to LRP. Further evidence that Factor VIII light chain binds to LRP was obtained by surface plasmon resonance analysis. This analysis revealed that isolated Factor VIII light chain and the intact Factor VIII heterodimer have a similar affinity for LRP. In addition, $^{125}$I-labeled Factor VIII heterodimer and Factor VIII light chain are degraded at a similar rate by LRP-expressing CHO-cells (Figure 2). These findings are in favor of the view that the Factor VIII light chain contributes to the interaction with LRP to a major extent.

Further support that the major binding site for LRP is located on the Factor VIII light chain is provided by the properties of the anti-Factor VIII monoclonal antibody ESH4. This antibody dose-dependently interferes with the binding of Factor VIII light chain to LRP (Lenting et al. 1999a). Antibody ESH4 has previously been identified to bind to the C2 domain region of Factor VIII light chain, which would be compatible with a LRP-interactive site within this part of the light chain. Indeed, a recombinant fragment encompassing the Factor VIII C2 domain displayed dose-dependent binding to LRP, the association of which was inhibited in the presence of ESH4.

With regard to LRP, the location of the Factor VIII light chain interaction sites was elucidated by using recombinant LRP fragments (Neels et al. 1999). These fragments contain the LRP LDLRA domains and are referred to as LRP-Clusters I to IV. Each of the LRP-Clusters was tested for binding to Factor VIII light chain. LRP-Clusters II and IV display efficient association to Factor VIII light chain, whereas no binding was observed employing LRP-Clusters I and III. From more extended analysis using overlapping fragments of LRP-Cluster II, we demonstrated that the Factor VIII light chain-interaction site comprises LDLRA domains 3 to 7 (Neels et al. 1999).

In conclusion, these findings are consistent with a model in which the carboxyterminal C2 domain of Factor VIII light chain comprises a site that may be recognized by two distinct regions within LRP.

Regions Involved in LRP/Factor VIII Complex Assembly: Factor VIII Heavy Chain

In initial studies, in which binding of Factor VIII heavy chain to immobilized LRP was assessed, no association could be observed (Lenting et al. 1999a). However, more recent studies in which a soluble ligand binding fragment of LRP, i.e. LRP-Cluster II, was incubated with immobilized Factor VIII heavy chain, demonstrated that Factor VIII heavy chain has the ability to bind this recombinant LRP fragment (unpublished). This indicates that Factor VIII heavy chain may also comprise a binding site for LRP. The notion that Factor VIII heavy chain may interact with LRP agrees with observations made by Saenko and coworkers, who reported that binding of purified intact Factor VIII to immobilized LRP is inhibited in the presence of Factor VIII heavy chain or a fragment thereof, Factor VIII A2 domain (Saenko et al. 1999). Furthermore, $^{125}$I-labeled
A2 domain is degraded approximately 5-fold less efficient by LRP-deficient mouse fibroblasts compared to LRP-expressing mouse fibroblasts. The exact location of the LRP binding site within the A2 domain was revealed using synthetic peptides. One of these peptides, encompassing the A2 domain residues 484-508, interferes with the binding of the A2 domain or Factor VIII to LRP, strongly suggesting that this particular Factor VIII region is involved in LRP binding (Saenko et al. 1999). Collectively, these data indicate that multiple sites within Factor VIII contribute to the interaction with LRP: one region encompasses the Factor VIII A2 domain residues 484-508, while a second region involves the carboxyterminal C2 domain of Factor VIII light chain (Figure 1). It is of interest to note that both these regions are major targets for inhibitory antibodies, which develop in approximately 20 % of the hemophilia A patients who are treated by replacement therapy with purified Factor VIII concentrates (Lollar 1999). Whether or not the development of such inhibitors involves a LRP-dependent mechanism remains speculative, and needs further studies. Another aspect of both the A2 and C2 domain regions is that they are known to serve an important role in Factor VIII function. The Factor VIII C2 domain comprises binding sites for vWF and negatively-charged phospholipids (Foster et al. 1990; Saenko et al. 1994), whereas the Factor VIII A2 domain region 484-508 has recently been shown to encompass a Factor IXa-interactive site (Fay and Scandella 1999) (Figure 1). Therefore, it seems likely that binding to LRP is incompatible with Factor VIII cofactor function.

**LRP/Factor VIII Complex Assembly: Effect of von Willebrand Factor**

In plasma, Factor VIII is tightly associated with von Willebrand Factor (vWF), its physiological carrier-protein (Sadler 1998). The importance of complex formation between Factor VIII and vWF is exemplified by patients having severe von Willebrand disease (type 3) who are lacking detectable vWF protein. Apart from exhibiting a defect in primary hemostasis due to the absence of vWF, these patients also have a secondary deficiency of Factor VIII. In addition, the half-life of intravenously administered Factor VIII in these patients is severely reduced (Sadler 1998). A similar phenotype is observed in patients having mutations in Factor VIII (tyrosine 1680) (Kemball-Cook and Tuddenham 1997) or vWF (von Willebrand disease Normandy, *i.e.* type 2N) (Nishino et al. 1989), which affect Factor VIII/vWF complex assembly. Despite normal levels of circulating vWF, Factor VIII levels in these patients are severely reduced. Apparently, association of Factor VIII to vWF is required to maintain appropriate levels of Factor VIII in the circulation, suggesting that vWF prevents Factor VIII from premature clearance. This effect of vWF is also observed in *in vitro* experiments using CHO-cell lines that express recombinant Factor VIII (Kaufman et al. 1988; Wise et al. 1991). Levels of Factor VIII that accumulate in medium are markedly increased in the presence of vWF, which may indicate that vWF prevents cellular internalization of Factor VIII.

In our studies, the effect of vWF on the association of Factor VIII to LRP was investigated. It appeared that vWF interfered efficiently with Factor VIII/LRP complex assembly in a dose-dependent manner. Binding of Factor VIII to LRP was inhibited by more than 90 % in the presence of a 10-fold molar excess of vWF over Factor VIII.
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(Lenting et al. 1999a). Furthermore, cellular degradation of Factor VIII was fully suppressed in the presence of vWF. It seems conceivable therefore, that vWF prevents Factor VIII binding to LRP. In this respect, it should be mentioned however that our results are dissimilar to those reported by Saenko and coworkers. In their studies, vWF was unable to interfere with binding of $^{125}\text{I}$-labeled Factor VIII to immobilized LRP, while cellular degradation of Factor VIII in the presence of vWF was reduced by approximately 50\% (Saenko et al. 1999). The reason for this discrepancy between these studies is unclear, but may be related to the different experimental approaches and cell lines that have been used.

The involvement of LRP in the clearance of Factor VIII in vivo has recently been studied by employing mice genetically deficient for vWF, which have a secondary deficiency of Factor VIII (20-30\% of normal) (Schwarz et al. In Press). As expected, endogenous Factor VIII levels were increased 2-3 fold upon the administration of vWF. Intriguingly, a similar increase of endogenous Factor VIII levels was observed upon the infusion of the LRP-antagonist RAP. Furthermore, pre-administration of RAP resulted in a delayed clearance of Factor VIII after its infusion. Apparently, the presence of RAP affects both endogenous and exogenous Factor VIII levels in the absence of vWF. These data are consistent with a mechanism in which inhibition of a RAP-sensitive pathway, most likely involving LRP, prevents the accelerated clearance of Factor VIII in the absence of vWF. Both the in vitro and in vivo studies may explain the beneficial effect of vWF on the in vivo survival of Factor VIII, in that vWF seems to interfere with LRP-mediated internalization of Factor VIII.

Activated Factors VIII and IX as Ligands for LRP

The observation that cellular degradation of Factor VIII is fully suppressed in the presence of vWF, leaves the role of LRP to those conditions in which Factor VIII is not associated to vWF. First, this may be under pathological conditions such as severe von Willebrand disease. Second, Factor VIII dissociates from vWF upon thrombin-mediated activation (reviewed by Lenting et al. 1998). Within activated Factor VIII (Factor VIIIa), the aminoterminal $a3$ segment of Factor VIII light chain has been removed. This results in loss of high affinity binding to vWF (Lollar et al. 1988), whereas the affinity for LRP remains unchanged (Lenting et al. 1999a). Dissociation of the Factor VIII/vWF complex allows Factor VIIIa to assemble into a membrane-bound active complex with its enzyme Factor IXa. The interaction between Factor IXa and Factor VIIIa is mediated by multiple interactive sites, located in both the heavy and light chain of Factor VIIIa (Fig. 1; (Mertens et al. 1999)).

A striking observation in this respect relates to our studies concerning the interaction between LRP and Factor IXa (Lenting et al. 1999b). Factor IXa efficiently binds to LRP, in contrast to its inactive precursor the Factor IX zymogen, in a reversible and dose- and calcium-dependent manner. In addition, the amount of Factor IXa degraded by LRP-deficient cells is approximately 35\% lower as compared to LRP-expressing cells, showing that LRP contributes to the binding and transport of Factor IXa to the intracellular degradation pathway. Thus, LRP is not only able to bind and internalize the cofactor Factor VIIIa, but also its enzyme Factor IXa. Whether LRP is able to bind both Factor VIIIa and Factor IXa simultaneously or to promote dissociation of the complex

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remains to be investigated. It is noteworthy that Factor VIII light chain has similar
affinity for both LRP-Cluster II and IV (K_d = 120 and 90 nM, respectively), whereas
Factor IXa displays 4-fold higher affinity for LRP-Cluster IV as compared to Cluster II
(K_d = 230 and 50 nM, respectively). This may allow a mechanism in which LRP
interacts with both Factor VIII light chain and Factor IXa simultaneously with its
Cluster II and IV, respectively.

The Factor Villa/Factor IXa complex is responsible for the conversion of Factor X into
its activated form, Factor Xa (reviewed by Lenting et al. 1998). It would be of interest
to investigate to what extent Factor Xa generation by the Factor VIIIa/Factor IXa
complex is affected by LRP. From previous studies it is known that LRP is abundantly
expressed at the surface of cells which are present at sites of vascular injury, like
monocytes, fibroblast and smooth muscle cells (Moestrup et al. 1992). The amount of
Factor Xa generated by the Factor VIIIa/Factor IXa complex at the surface of
endothelial cells is markedly higher as compared to the amount generated at the surface
of monocytes or fibroblasts (Brinkman et al. 1994). This agrees with the fact that human
umbilical vein endothelial cells lack LRP (Grobmyer et al. 1993), whereas monocytes
and fibroblasts constitutively express this receptor at their surface (Moestrup et al.
1992). Therefore, it seems conceivable that LRP interferes with the activity of the
Factor VIIIa/Factor IXa complex, resulting in down-regulation of Factor Xa generation.

Initiating and Propagating Steps of the Coagulation Process:
Modulation by LRP?

Upon vascular injury, various hemostatic pathways react in a concerted action in order
to arrest bleeding. One of these pathways, the coagulation cascade, is a sequence of
amplifying, often cell-surface dependent reactions in which zymogens are converted
into their enzymatic conformations. This cascade ultimately results in the generation of
thrombin, which is responsible for the formation of an insoluble fibrin-network which
stabilizes the primary hemostatic plug. Traditionally, two distinct coagulation pathways
have been distinguished: the extrinsic and intrinsic pathway (Figure 3). However,
extensive research in the area of coagulation revealed the presence of several
biochemical connections between both pathways (Mann 1999). The extrinsic pathway
rather represents an initial phase mediated by the Tissue Factor/Factor VIIa complex,
while the intrinsic pathway represent an amplifying loop involving the Factor
VIIIa/Factor IXa complex.

The coagulation cascade is tightly regulated by a complex system of feed-back reactions
and specific inhibitors, including those of the serine protease inhibitor (serpin) and
Kunitz-type inhibitor family. These inhibitors form irreversible complexes with their
target-enzymes, and such complexes (e.g. thrombin/antithrombin and Factor Xa/α2-
macroglobulin complexes) may subsequently be removed from the circulation by a
mechanism that involves LRP (Strickland and Kounnas 1997; Narita et al. 1998),
illustrating that LRP and the coagulation system are linked in a functional manner.
However, removal of inactive complexes is unlikely to provide a significant
contribution to the regulation of coagulation. In contrast, the notion that LRP is able to
remove the active proteins Factor VIIIa and Factor IXa and not the inactive vWF-bound
Factor VIII and the zymogen Factor IX underscores a possible regulatory role for LRP
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in blood coagulation (Figure 3). This view becomes even more intriguing considering an elegant study on the interaction between LRP and the Tissue Factor/Factor VIIa complex, recently reported by Hamik and coworkers (Hamik et al. 1999). This complex acts as a trigger of the coagulation cascade when formed at the surface of various cells, including endothelial cells and monocytes. The activity of this complex is inhibited in a reversible manner by the Kunitz-type inhibitor Tissue Factor Pathway Inhibitor (TFPI) (Broze, Jr. 1992), which has previously been established to be a ligand for LRP (Warshawsky et al. 1994). Hamik and coworkers showed that in the presence of TFPI the amount of Tissue Factor/Factor VIIa complex present at the surface of monocytes was decreased. This down-regulation, however, was efficiently abrogated when performed in the presence of the LRP-antagonist RAP. This indicates that the trimeric Tissue Factor/Factor VIIa/Tissue Factor Pathway Inhibitor complex is internalized via a LRP-mediated pathway. Indeed, recombinant Tissue Factor Pathway Inhibitor variants lacking the LRP-binding region were unable to down-regulate Tissue Factor/Factor VIIa complexes at the monocyte cell surface. More recently, it has been reported that a similar mechanism exists at the surface of fibroblasts (Iakhiaev et al. 1999). It is relevant to note that down-regulation at the surface of fibroblasts requires the presence of the serine protease Factor Xa, whereas at the surface of monocytes down-regulation proceeds efficiently in the absence of Factor Xa. Thus, subtle differences in mechanisms may exist between various cell types.

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Fig. 3: Schematic representation of the coagulation cascade. Depicted is a simplified scheme of the activation reactions within the coagulation cascade. Vascular damage allows formation of the Tissue Factor/Factor VIIa complex at cellular surfaces. This complex initiates the activation of small amounts of Factor IX and Factor X, as the complex is rapidly inhibited by TFPI in a reversible manner. Factor Xa subsequently generates small amounts of thrombin, an enzyme essential for platelet activation and fibrin polymerisation. Feedback activation mediated by thrombin (dotted lines) results in activation of Factors XI, VIII and V, allowing formation of Factor IXa/Factor VIIIa and Factor Xa/Factor Va complexes. These complexes are responsible for a further boost of thrombin formation ("propagation step"). Within this propagation step, Factor VIIIa and Factor IXa are identified as targets for LRP, and LRP may therefore contribute to down-regulation of this pathway. LRP is also involved in down-regulation of the initiation step by removal of the trimeric Tissue Factor/Factor VIIa/TFPI complex from the cellular surface.
Concluding remarks

In conclusion, compelling evidence has emerged that shows that LRP is able to bind and internalize a number of components that are critical for appropriate coagulation. As these components are related to both initiating (Tissue Factor/Factor VIIa/Tissue Factor Pathway Inhibitor complex) and propagating (Factor VIIIa/Factor IXa complex) stages of the coagulation process, it is tempting to speculate that LRP serves a sofar unrecognized role in modulation of the coagulation cascade. In addition, both the in vitro and in vivo studies (Lenting et al. 1999; Saenko et al. 1999; Schwarz et al. In Press) may provide an explanation for the longstanding observation that the circulatory lifetime of Factor VIII in patients with severe von Willebrand disease is decreased. Thus, LRP may contribute to the circulating levels of proteins involved in coagulation. Finally, the observation that LRP is involved in cellular signalling indicates that the coagulation-related proteins have the potential to use LRP to induce cellular signalling processes. In our view, this possibility is challenging and deserves further study.

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References


Chapter 5


Modulatory Role for LRP in Coagulation


